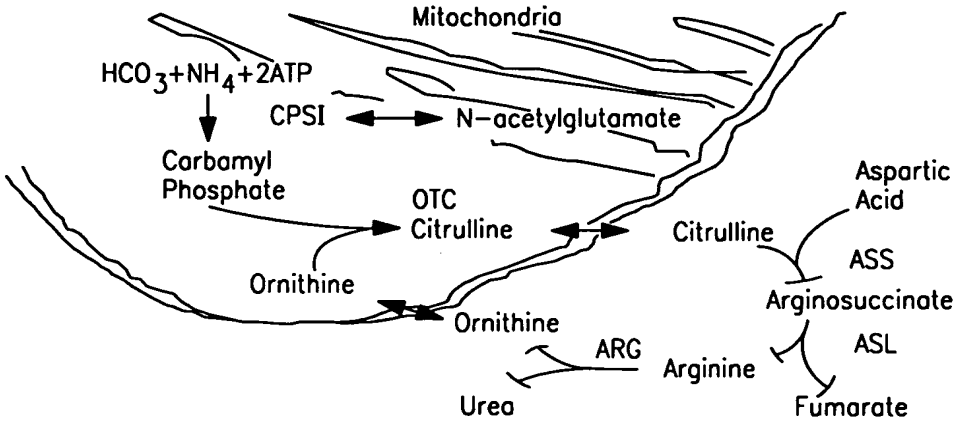

Mitochondria
HCO_3+NH_4+2ATP
CPSI
N-acetylglutamate
Carbamyl Phosphate
OTC
Citrulline
Ornithine
Aspartic Acid
Citrulline
ASS
Arginosuccinate
Ornithine
ASL
ARG
Arginine
Urea
Fumarate

FIG. 1

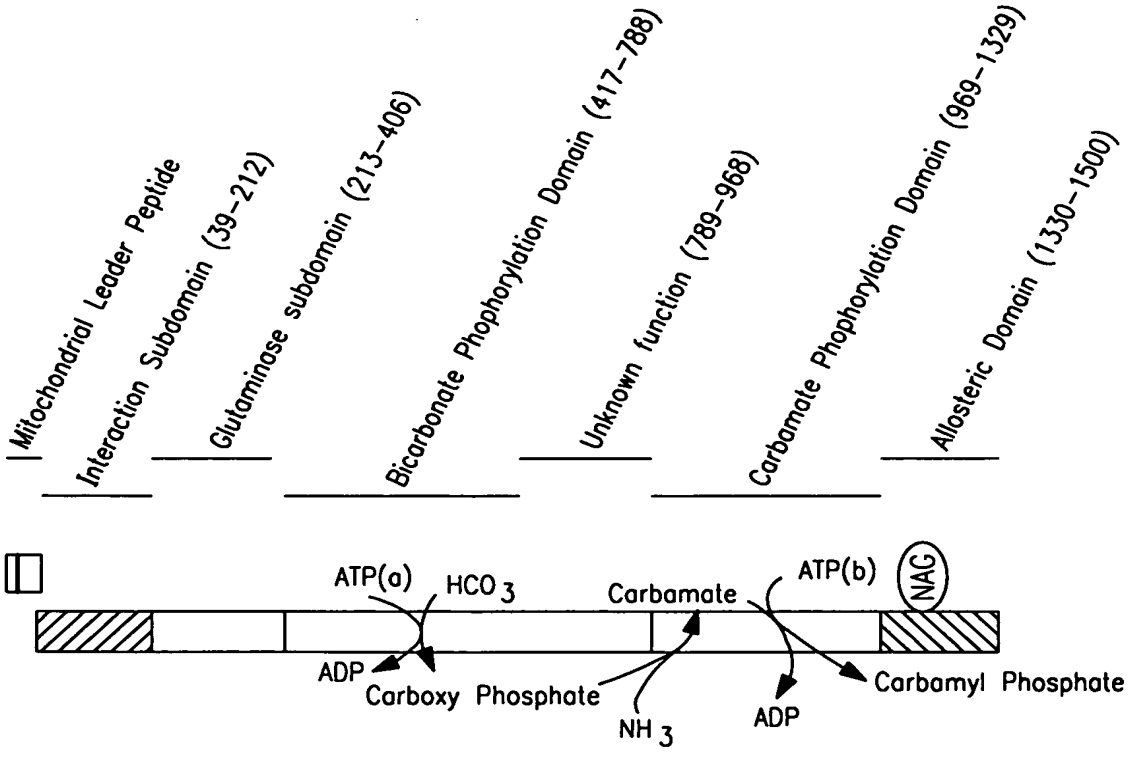

Mitochondrial Leader Peptide
Interaction Subdomain (39-212)
Glutaminase subdomain (213-406)
Bicarbonate Phophorylation Domain (417-788)
Unknown function (789-968)
Carbamate Phophorylation Domain (969-1329)
Allosteric Domain (1330-1500)
ATP(a)
HCO_3
Carbamate
ATP(b)
NAG
ADP
Carboxy Phosphate
NH_3
ADP
Carbamyl Phosphate

FIG. 2

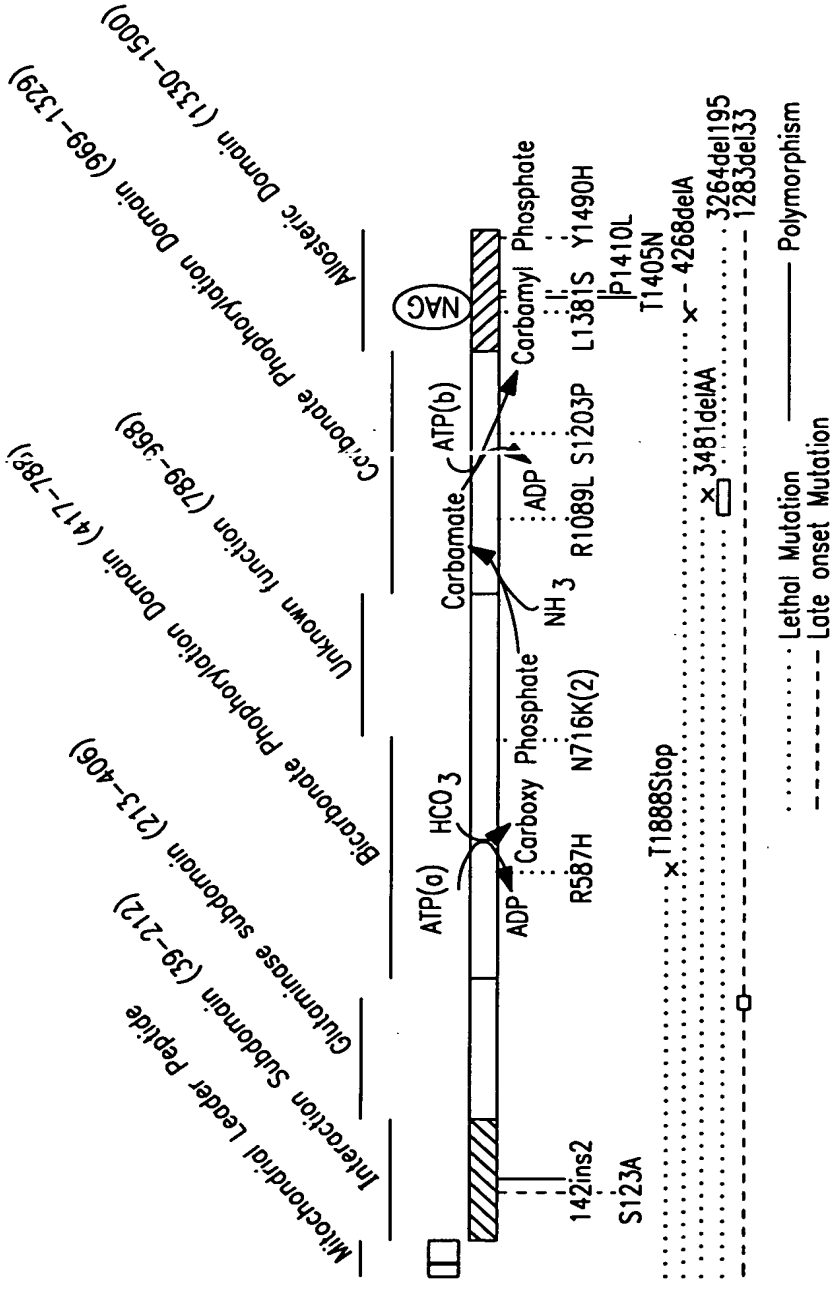

Mitochondrial Leader Peptide
Interaction Subdomain (39-212)
Glutaminase subdomain (213-406)
Bicarbonate Phophonylation Domain (417-788)
Unknown function (789-968)
Carbonate Phophonylation Domain (969-1329)
Allosteric Domain (1330-1500)
NAG
ATP(b)
Carbamyl Phosphate
ADP
Carbamate
NH3
ATP(a)
HCO3
Carboxy Phosphate
ADP
R1089L S1203P L1381S Y1490H
P1410L
T1405N
4268delA
3481delAA
3264del195
1283del33
R587H N716K(2)
T1888Stop
142ins2
S123A
Lethal Mutation
Late onset Mutation
Polymorphism

FIG. 3

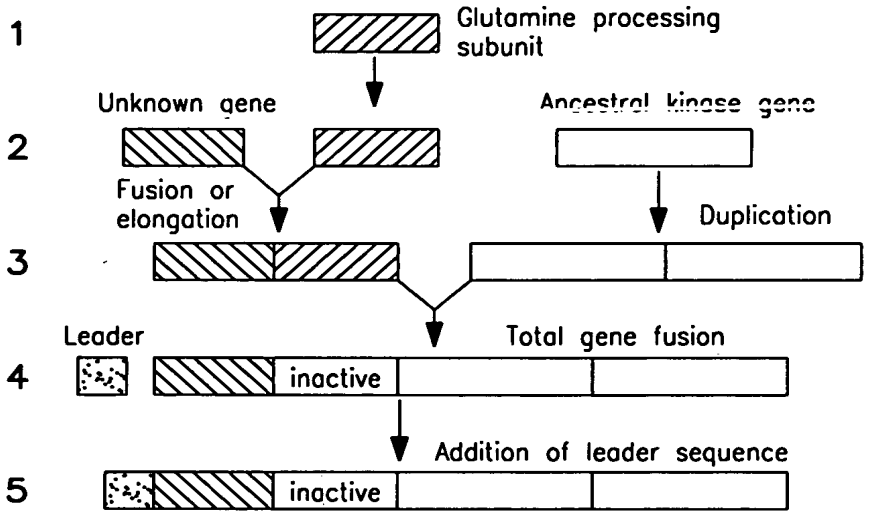

1
Glutamine processing subunit
Unknown gene
Ancestral kinase gene
2
Fusion or elongation
Duplication
3
Leader
Total gene fusion
4
inactive
Addition of leader sequence
5
inactive

FIG. 4

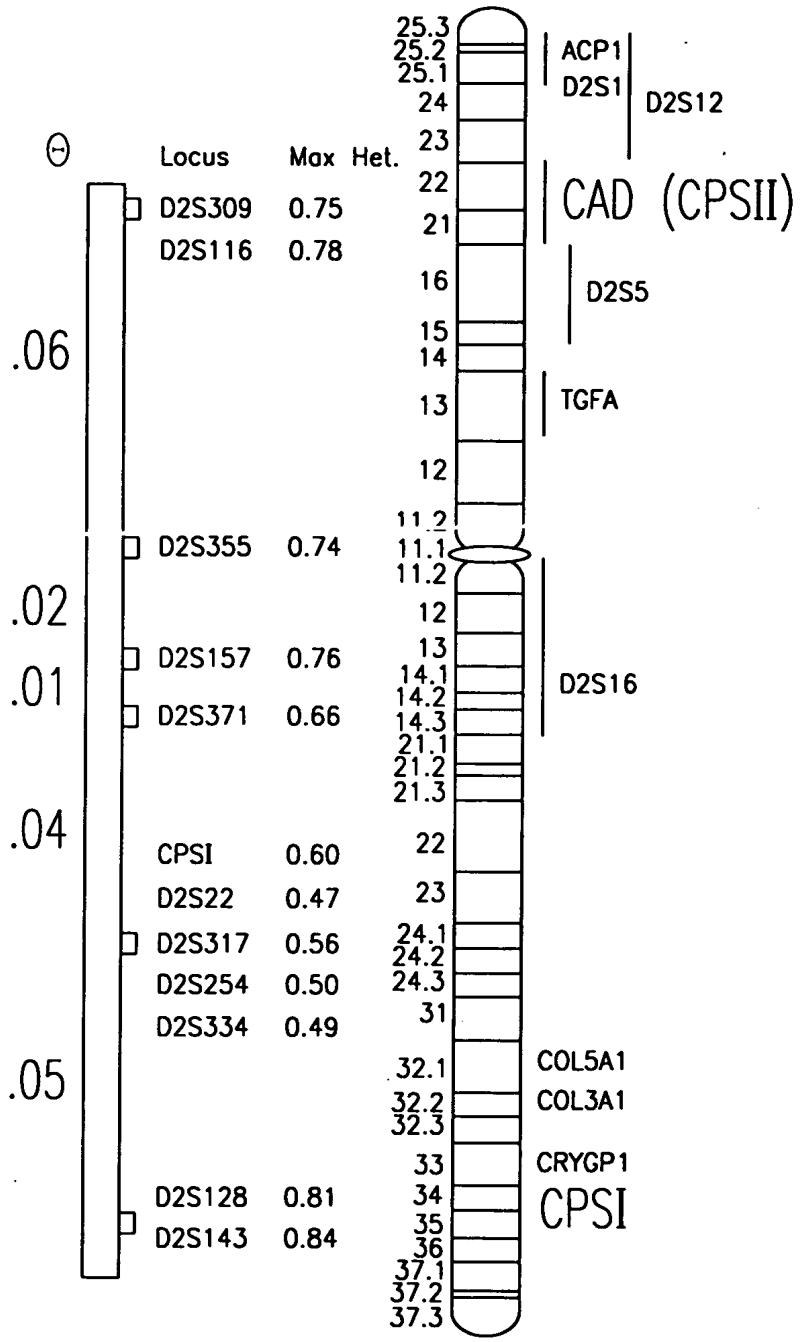

Θ
Locus
Max Het.
D2S309 0.75
D2S116 0.78
D2S355 0.74
D2S157 0.76
D2S371 0.66
CPSI 0.60
D2S22 0.47
D2S317 0.56
D2S254 0.50
D2S334 0.49
D2S128 0.81
D2S143 0.84
.06
.02
.01
.04
.05
25.3
25.2
25.1
24
23
22
21
16
15
14
13
12
11.2
11.1
11.2
12
13
14.1
14.2
14.3
21.1
21.2
21.3
22
23
24.1
24.2
24.3
31
32.1
32.2
32.3
33
34
35
36
37.1
37.2
37.3
ACP1
D2S1
D2S12
CAD (CPSII)
D2S5
TGFA
D2S16
COL5A1
COL3A1
CRYGP1
CPSI

FIG. 5

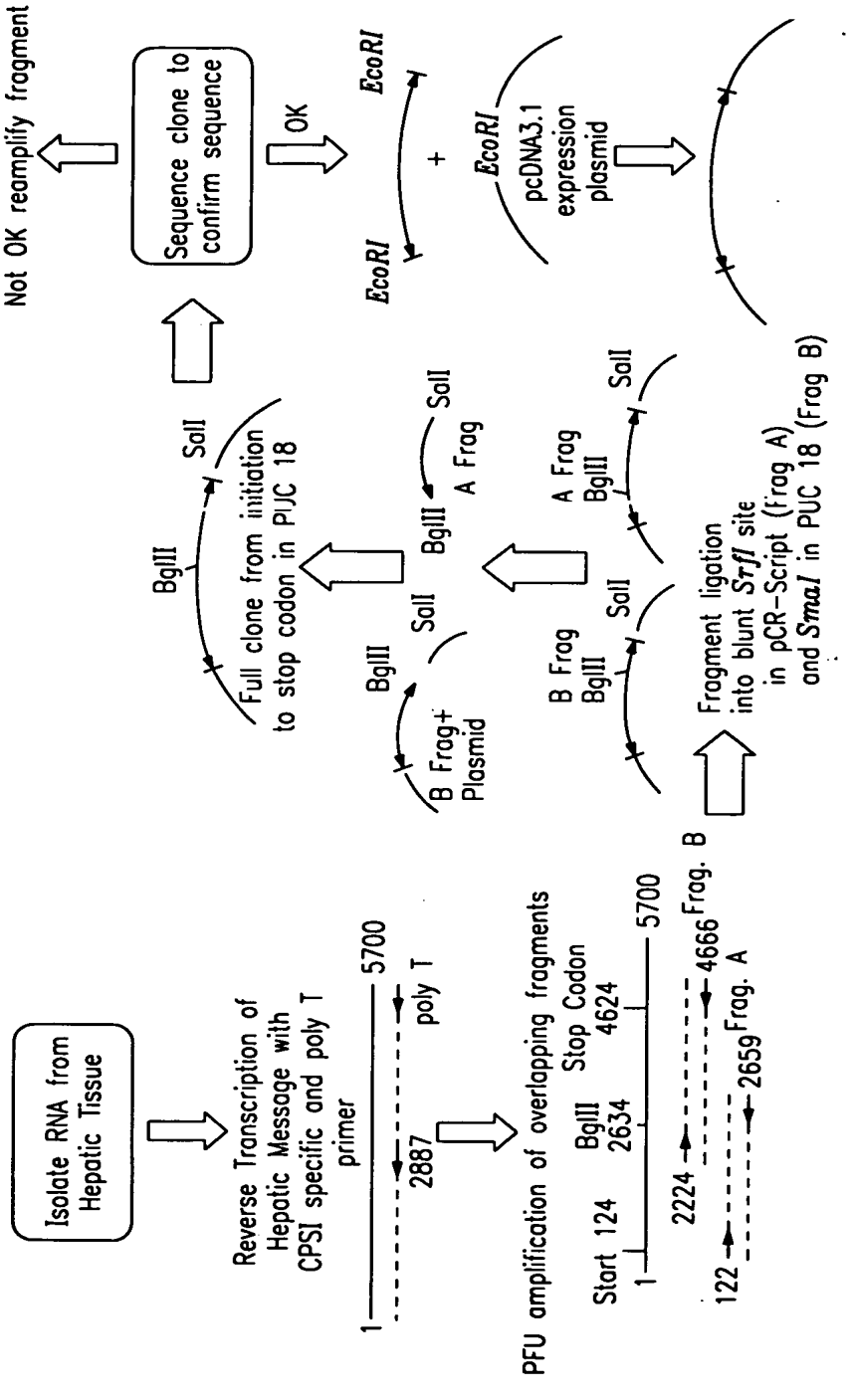

Isolate RNA from Hepatic Tissue
Reverse Transcription of Hepatic Message with CPSI specific and poly T primer
1
5700
2887
poly T
PFU amplification of overlapping fragments
Start 124
BglII 2634
Stop Codon 4624
5700
1
2224
4666
Frag. B
122
2659
Frag. A
Fragment ligation into blunt SrfI site in pCR-Script (Frag A) and SmaI in PUC 18 (Frag B)
B Frag BglII
SalI
A Frag BglII
SalI
B Frag+ Plasmid
BglII
SalI
BglII
A Frag
SalI
Full clone from initiation to stop codon in PUC 18
BglII
SalI
Sequence clone to confirm sequence
Not OK reamplify fragment
OK
EcoRI
EcoRI
+
EcoRI
pcDNA3.1 expression plasmid

FIG. 6

Title: Therapeutic Methods
Employing Nitric Oxide Precursors
Inventors: Summar et al.
Attorney Docket No. 1242/58

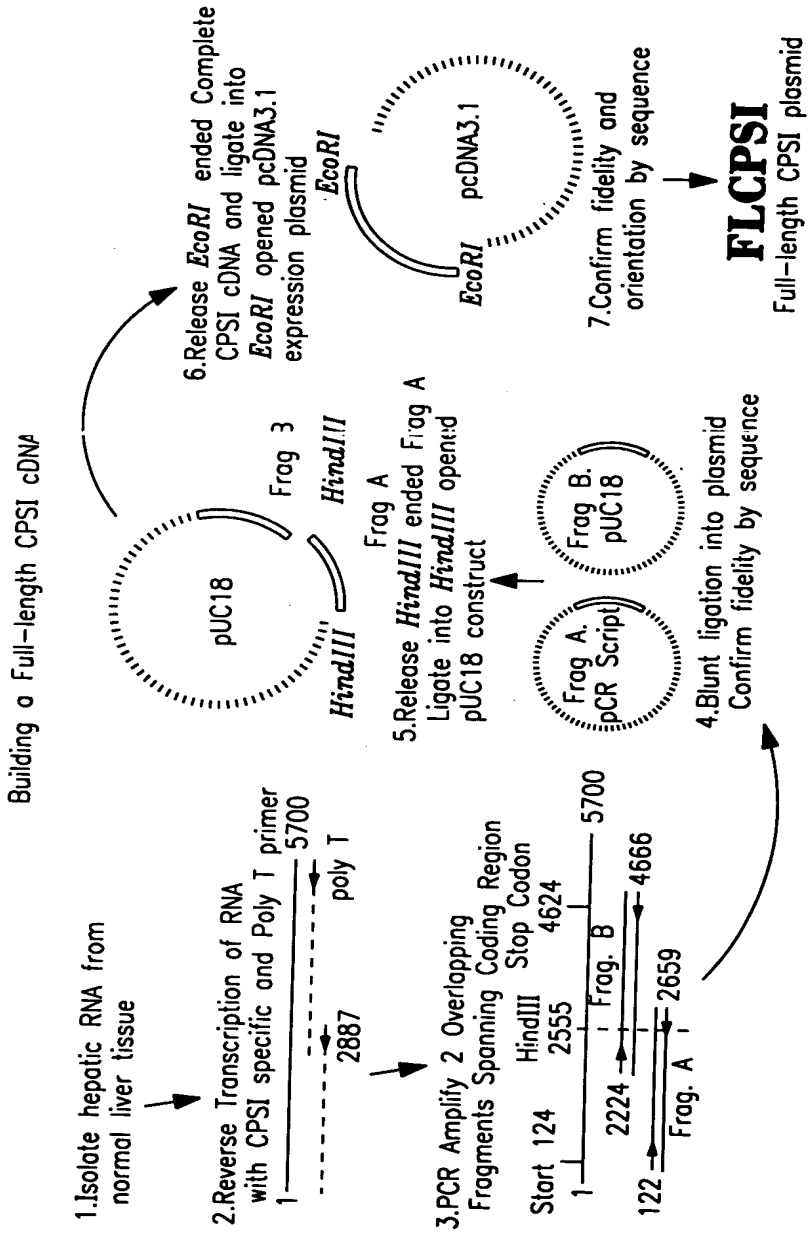


FIG. 7

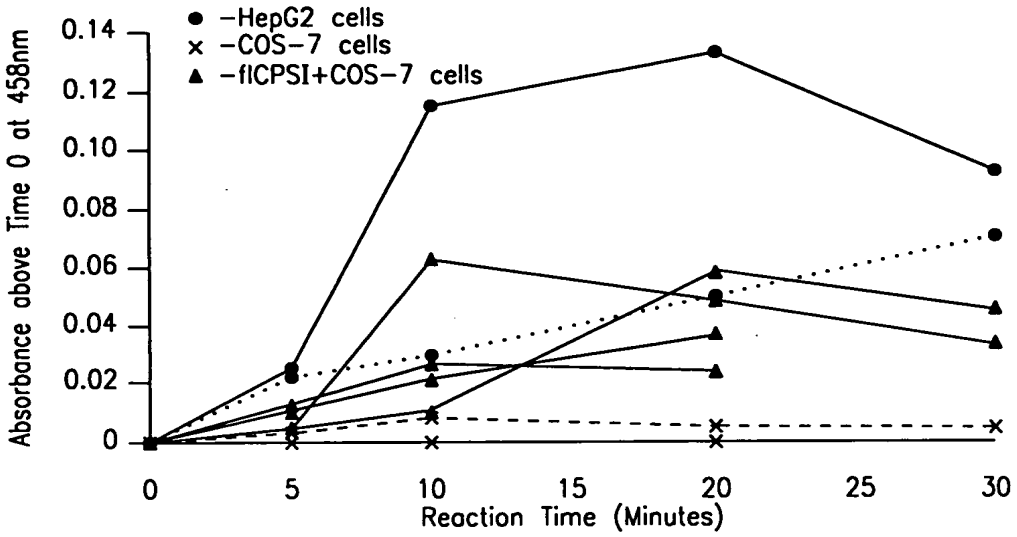

● -HepG2 cells
× -COS-7 cells
▲ -flCPSI+COS-7 cells
Absorbance above Time 0 at 458nm
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0
0
5
10
15
20
25
30
Reaction Time (Minutes)

FIG. 8

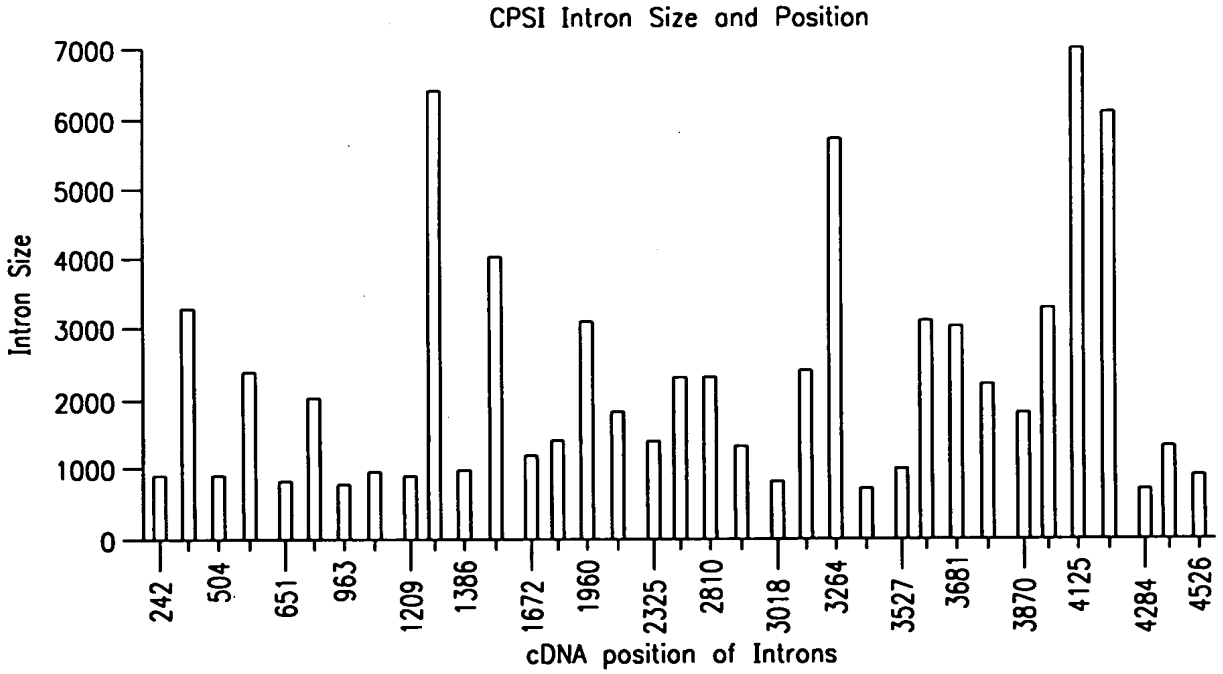

CPSI Intron Size and Position
Intron Size
7000
6000
5000
4000
3000
2000
1000
0
242
504
651
963
1209
1386
1672
1960
2325
2810
3018
3264
3527
3681
3870
4125
4284
4526
cDNA position of Introns

FIG. 9

```
1
ctacttctca tgttcagcaa tttcttcttc tttatgtttt aaattacatg ttccataaaa ataagaaat
71
cactgtgata cggtaattga ttttttcatt ttaaatgcag/(intron exon boundary)
111        (U4295)
CTGTTTGCCA CGGAAGCCAC ATCAGACTGG CTCAACGCCA ACAATGTCCC TGCCACCCCA GTGGCATGGC
181
CGTCTCAAGA AGGACAGAAT CCCAGCCTCT CTTCCATCAG AAA/ (intron exon boundary)
224                     GTCGGAGA GAAGGTAGTC TT    L(135a)
gtaagaacta ggcatactgt tttctgaaat aatttagagg attaactttg agaaccagta tatgaatatt
294
caccttgctt gattgcaagt cttttaaaac aaatttaaaa atgaatacat ttgtggatga ttgtcaagtt
364                                                (L135b)
tcactctcca tcactatgga atacataacg tcatgtgtac atggtgatat gaaacgtgtt tcaaaatact
434
tcttagtaag gatactttcc ttgacggaaa caagtgagag tatgaagaat gtaatgcagc ac
```

Primer	Begins	Size	SEQ ID NO:
U4295	119	20	8
L135a	220	21	9
L135b	370	24	10
Spanner 1	agctgtttgccacggaagcc		6
Spanner 2	cccagcctctcttccatcagaaagtaag		7

Pairs
U4295 - L135a 101 base fragment

U4295 - L135b 251 base fragment

Spanner1 - Spanner2 119 base fragment

FIG. 10

CPSI T1405 SEQUENCE (SEQ ID NO:4)

MTRILTAFKV VRTLKTGFGF TNVTAHQKWK FSRPGIRLLS VKAQTAHIVL EDGTKMKGYS
FGHPSSVAGE VVFNTGLGGY PEAITDPAYK GQILTMANPI IGNGGAPDTT ALDELGLSKY
LESNGIKVSG LLVLDYSKDY NHWLATKSLG QWLQEEKVPA IYGVDTRMLT KIIRDKGTML
GKIEFEGQPV DFVDPNKQNL IAEVSTKDVK VYGKGNPTKV VAVDCGIKNN VIRLLVKRGA
EVHLVPWNHD FTKMEYDGIL IAGGPGNPAL AEPLIQNVRK ILESDRKEPL FGISTGNLIT
GLAAGAKTYK MSMANRGQNQ PVLNITNKQA FITAQNHGYA LDNTLPAGWK PLFVNVNDQT
NEGIMHESKP FFAVQFHPEV TPGPIDTEYL FDSFFSLIKK GKATTITSVL PKPALVASRV
EVSKVLILGS GGLSIGQAGE FDYSGSQAVK AMKEENVKTV LMNPNIASVQ TNEVGLKQAD
TVYFLPITPQ FVTEVIKAEQ PDGLILGMGG QTALNCGVEL FKRGVLKEYG VKVLGTSVES
IMATEDRQLF SDKLNEINEK IAPSFAVESI EDALKAADTI GYPVMIRSAY ALGGLGSGIC
PNRETLMDLS TKAFAMTNQI LVEKSVTGWK EIEYEVVRDA DDNCVTVCNM ENVDAMGVHT
GDSVVVAPAQ TLSNAEFQML RRTSINVVRH LGIVGECNIQ FALHPTSMEY CIIEVNARLS
RSSALASKAT GYPLAFIAAK IALGIPLPEI KNVVSGKTSA CFEPSLDYMV TKIPRWDLDR
FHGTSSRIGS SMKSVGEVMA IGRTFEESFQ KALRMCHPSI EGFTPRLPMN KEWPSNLDLR
KELSEPSSTR IYAIAKAIDD NMSLDEIEKL TYIDKWFLYK MRDILNMEKT LKGLNSESMT
EETLKRAKEI GFSDKQISKC LGLTEAQTRE LRLKKNIHPW VKQIDTLAAE YPSVTNYLYV
TYNGQEHDVN FDDHGMMVLG CGPYHIGSSV EFDWCAVSSI RTLRQLGKKT VVVNCNPETV
STDFDECDKL YFEELSLERI LDIYHQEACG GCIISVGGQI PNNLAVPLYK NGVKIMGTSP
LQIDRAEDRS IFSAVLDELK VAQAPWKAVN TLNEALEFAK SVDYPCLLRP SYVLSGSAMN
VVFSEDEMKK FLEEATRVSQ EHPVVLTKFV EGAREVEMDA VGKDGRVISH AISEHVEDAG
VHSGDATLML PTQTISQGAI EKVKDATRKI AKAFAISGPF NVQFLVKGND VLVIECNLRA
SRSFPFVSKT LGVDFIDVAT KVMIGENVDE KHLPTLDHPI IPADYVAIKA PMFSWPRLRD
ADPILRCEMA STGEVACFGE GIHTAFLKAM LSTGFKIPQK GILIGIQQSF RPRFLGVAEQ
LHNEGFKLFA TEATSDWLNA NNVPATPVAW PSQEGQNPSL SSIRKLIRDG SIDLVINLPN
NNTKFVHDNY VIRRTAVDSG IPLLTNFQVT KLFAEAVQKS RKVDSKSLFH YRQYSAGKAA
X

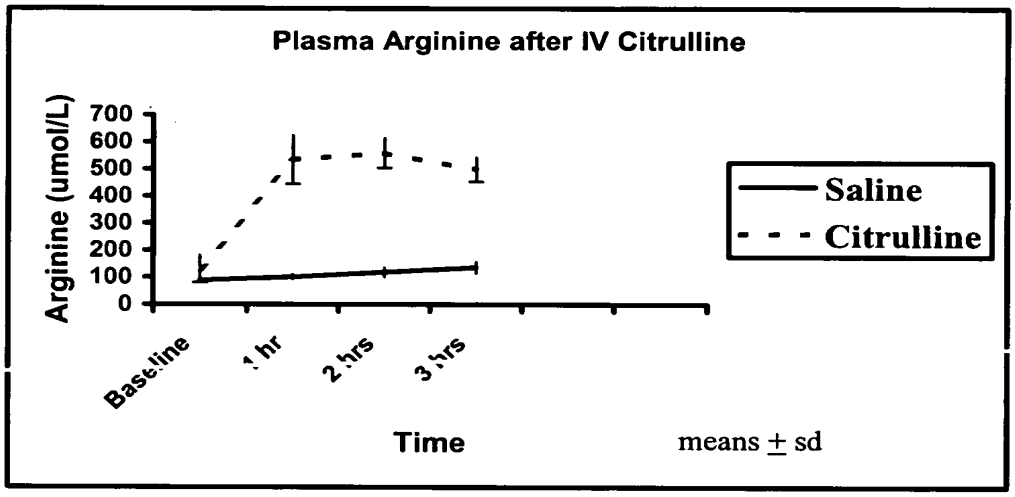
FIG. 11

CPSI N1405 SEQUENCE (SEQ ID NO:2)

MTRILTAFKV VRTLKTGFGF TNVTAHQKWK FSRPGIRLLS VKAQTAHIVL EDGTKMKGYS
FGHPSSVAGE VVFNTGLGGY PEAITDPAYK GQILTMANPI IGNGGAPDTT ALDELGLSKY
LESNGIKVSG LLVLDYSKDY NHWLATKSLG QWLQEEKVPA IYGVDTRMLT KIIRDKGTML
GKIEFEGQPV DFVDPNKQNL IAEVSTKDVK VYGKGNPTKV VAVDCGIKNN VIRLLVKRGA
EVHLVPWNHD FTKMEYDGIL IAGGPGNPAL AEPLIQNVRK ILESDRKEPL FGISTGNLIT
GLAAGAKTYK MSMANRGQNQ PVLNITNKQA FITAQNHGYA LDNTLPAGWK PLFVNVNDQT
NEGIMHESKP FFAVQFHPEV TPGPIDTEYL FDSFFSLIKK GKATTITSVL PKPALVASRV
EVSKVLILGS GGLSIGQAGE FDYSGSQAVK AMKEENVKTV LMNPNIASVQ TNEVGLKQAD
TVYFLPITPQ FVTEVIKAEQ PDGLILGMGG QTALNCGVEL FKRGVLKEYG VKVLGTSVES
IMATEDRQLF SDKLNEINEK IAPSFAVESI EDALKAADTI GYPVMIRSAY ALGGLGSGIC
PNRETLMDLS TKAFAMTNQI LVEKSVTGWK EIEYEVVRDA DDNCVTVCNM ENVDAMGVHT
GDSVVVAPAQ TLSNAEFQML RRTSINVVRH LGIVGECNIQ FALHPTSMEY CIIEVNARLS
RSSALASKAT GYPLAFIAAK IALGIPLPEI KNVVSGKTSA CFEPSLDYMV TKIPRWDLDR
FHGTSSRIGS SMKSVGEVMA IGRTFEESFQ KALRMCHPSI EGFTPRLPMN KEWPSNLDLR
KELSEPSSTR IYAIAKAIDD NMSLDEIEKL TYIDKWFLYK MRDILNMEKT LKGLNSESMT
EETLKRAKEI GFSDKQISKC LGLTEAQTRE LRLKKNIHPW VKQIDTLAAE YPSVTNYLYV
TYNGQEHDVN FDDHGMMVLG CGPYHIGSSV EFDWCAVSSI RTLRQLGKKT VVVNCNPETV
STDFDECDKL YFEELSLERI LDIYHQEACG GCIISVGGQI PNNLAVPLYK NGVKIMGTSP
LQIDRAEDRS IFSAVLDELK VAQAPWKAVN TLNEALEFAK SVDYPCLLRP SYVLSGSAMN
VVFSEDEMKK FLEEATRVSQ EHPVVLTKFV EGAREVEMDA VGKDGRVISH AISEHVEDAG
VHSGDATLML PTQTISQGAI EKVKDATRKI AKAFAISGPF NVQFLVKGND VLVIECNLRA
SRSFPFVSKT LGVDFIDVAT KVMIGENVDE KHLPTLDHPI IPADYVAIKA PMFSWPRLRD
ADPILRCEMA STGEVACFGE GIHTAFLKAM LSTGFKIPQK GILIGIQQSF RPRFLGVAEQ
LHNEGFKLFA TEATSDWLNA NNVPANPVAW PSQEGQNPSL SSIRKLIRDG SIDLVINLPN
NNTKFVHDNY VIRRTAVDSG IPLLTNFQVT KLFAEAVQKS RKVDSKSLFH YRQYSAGKAA
X

FIG. 12

FIGURE 13


Plasma Arginine after IV Citrulline
Arginine (umol/L)
700
600
500
400
300
200
100
0
Baseline
1 hr
2 hrs
3 hrs
Time
Saline
Citrulline
means ± sd